

U.S.S.N. 10/792,302
Filed: March 3, 2004
AMENDMENT AND RESPONSE TO OFFICE ACTION

In the Claims

1. (currently amended) A method for isolating a pluripotent or multipotent spore-like cell from a biological tissue or cell containing fluid, the method comprising (a) obtaining a tissue or cell containing fluid ~~that has been exposed and exposing it to an environment in which differentiated or partially differentiated cells in the tissue or fluid die, wherein the environment includes one or more conditions selected from the group consisting of~~ temperature of 42°C or greater, freezing, non-physiological salt concentration, essential absence of oxygen for at least four hours, size separation and passaging in cell culture, treatment with acid or base, radiation and drying, and (b) separating the viable spore-like cells from the dead differentiated or partially differentiated cells ~~that have died.~~
2. (currently amended) The method of claim 1, further comprising disrupting the tissue or fluid either before or after step (a) and separating the viable spore-like cell from the dead differentiated or partially differentiated cells by size separation.
3. (currently amended) The method of claim 2, ~~wherein disrupting the tissue or fluid comprises cutting the tissue into pieces, scraping the tissue with a blunt instrument, or passing the tissue or fluid through a series of devices having progressively smaller apertures~~ 1 wherein the spore-like cell fails to demonstrate activity in a microtetrastolium assay.
4. (currently amended) The method of claim 3, ~~wherein the devices are pipettes~~ 1 wherein the spore-like cells contain between approximately 50 and 90% by volume nuclear material.

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5. (currently amended) The method of claim 4, ~~wherein the smallest pipette has an inner bore 1 wherein the spore-like cells have a diameter of approximately 15 microns or less.~~
6. (currently amended) The method of claim 1 3, wherein the spore-like cells have a diameter of between 0.1 and 3.0 microns ~~devices are filters.~~
7. (currently amended) The method of claim 1 wherein the tissue or fluid is treated with salt, acid or base and the spore-like cells isolated ~~6, wherein the finest filter has a pore size of approximately 15 microns.~~
8. (original) The method of claim 1, wherein the biological tissue comprises a tissue that originates from the endoderm.
9. (original) The method of claim 1, wherein the biological tissue comprises a tissue that originates from the mesoderm.
10. (original) The method of claim 1, wherein the biological tissue comprises a tissue that originates from the ectoderm.
11. (original) The method of claim 1, wherein the biological fluid comprises blood, urine, or saliva.
12. (original) The method of claim 1, wherein the biological fluid is cerebrospinal fluid.
13. (original) The method of claim 1, wherein the environment is an oxygen-poor environment.
14. (original) The method of claim 1, wherein the environment is one in which the temperature is above or below the range of temperatures in which differentiated or partially differentiated cells can survive.
15. (original) The method of claim 1, wherein the environment contains a toxin or infectious agent that kills differentiated or partially differentiated cells.

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16. (currently amended) ~~A The method for isolating a spore-like cell from a biological tissue or fluid, the method comprising obtaining a tissue or fluid that has been exposed to an environment that is either 42-degree C., or more, or 0-degree C., or less, placing the tissue or fluid in a tissue culture vessel, allowing the spore-like cells to adhere to the vessel, and rinsing away non-spore-like cells, the tissue or fluid having been exposed to the environment without first being treated with a protective agent of claim 1 wherein the environment contains radiation or is dessicating.~~

17. (currently amended) The method of claim 16, ~~further comprising disrupting the tissue or fluid either before or after exposure to the environment or before or after placement in the tissue culture vessel~~ 1 further comprising placing the cells into a matrix for implantation into a site for tissue repair, augmentation or regeneration.

18. (currently amended) The method of claim 17, ~~wherein disrupting the tissue comprises cutting the tissue into pieces, scraping the tissue with a blunt instrument, or passing the tissue or fluid through a series of devices having progressively smaller apertures~~ further comprising implanting the matrix into a site for tissue repair, augmentation or regeneration.

19. (currently amended) The method of claim 18, ~~wherein the devices are pipettes~~ 1 further comprising culturing the spore-like cells.

20. (currently amended) The method of claim 14, ~~wherein the smallest pipette has an inner bore diameter of approximately 15 microns~~ 18 further comprising implanting the matrix into a tissue selected from the group consisting of the visual system, auditory system, nasal epithelium, alimentary canal, pancreas, gallbladder, bladder, kidney, liver, heart, respiratory system, nervous system, reproductive system, endocrine system,

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immune system, bone, muscle, tooth, nail, and skin.

21. (currently amended) The method of claim 18, ~~wherein the devices are filters~~ 17
wherein the matrix is a hydrogel.

22. (currently amended) The method of claim 21, ~~wherein the finest filter has a pore size~~
~~of approximately 15 microns~~ 1 wherein the tissue is obtained from a tissue selected from
the group consisting of cardiac, smooth and skeletal muscle, intestine, bladder, kidney,
liver, lung, adrenal gland, skin, retina, nasal epithelium, brain, spinal cord, periosteum,
perichondrium, fascia, and pancreas.

23. (currently amended) The method of claim 16, ~~wherein the biological tissue or fluid~~
~~originates from the endoderm~~ 1 wherein the spore-like cells are frozen after isolation.

24. (currently amended) The method of claim 16, ~~wherein the biological tissue or fluid~~
~~originates from the mesoderm~~ 1 further comprising inducing the isolated spore-like cells
to differentiate.

25. (currently amended) The method of claim 16, ~~wherein the biological tissue is a tissue~~
~~that originates from the ectoderm~~ 21 wherein the spore-like cells are introduced into a
support structure.

26. (currently amended) The method of claim 16, ~~wherein the biological fluid is blood,~~
~~urine, or saliva~~ 17 wherein the matrix is a porous polymer mesh, suture, film or sponge.

27. (currently amended) The method of claim 1 16, wherein the biological fluid is
cerebrospinal fluid.

28. (cancelled) The method of claim 16, wherein the environment is 0°C. or less and the
protective agent is a cryopreservative.

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29. (cancelled) The method of claim 16, wherein the tissue or fluid is intentionally exposed to the environment.